

WHAT WE CLAIM IS:

1. An isolated mutated GDF-9 nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:
 - a) SEQ ID NOs. 1, 3 or 5;
 - 5 b) a sequence complementary to the molecule defined in a);
 - c) a functional fragment or variant of the sequences in a) or b);
 - d) an anti-sense sequence to any of the molecules defined in a), b) or c).
2. An isolated mutated GDF-9B nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:
 - 10 a) SEQ ID NOs. 7, 9, 11, 13, 15 or 17;
 - b) a sequence complementary to the molecule defined in a)
 - c) an anti-sense sequence to any of the molecules defined in a) or b).
3. An isolated GDF-9 nucleic acid molecule comprising a mutation in at least one codon of the sequence associated with receptor binding and/or dimerisation.
- 15 4. An isolated GDF-9 nucleic acid molecule as claimed in claim 3, wherein said mutation results in an amino acid substitution in the polypeptide encoded by said nucleic acid sequence.
5. An isolated GDF-9 nucleic acid molecule as claimed in claim 4, wherein said amino acid substitution is present in a receptor binding domain and disrupts
20 receptor binding.

6. An isolated GDF-9 nucleic acid molecule as claimed in claim 4, wherein said amino acid substitution is present in a dimerisation domain and disrupts dimerisation.
7. An isolated GDF-9B nucleic acid molecule comprising a mutation in at least one codon sequence associated with receptor binding and/or dimerisation.
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8. An isolated GDF-9B nucleic acid molecule as claimed in claim 7, wherein said mutation results in an amino acid substitution in the polypeptide encoded by said nucleic acid sequence.
9. An isolated GDF-9B nucleic acid molecule as claimed in claim 8, wherein said amino acid substitution is present in a receptor binding domain and disrupts receptor binding.
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10. An isolated GDF-9B nucleic acid molecule as claimed in claim 8, wherein said amino acid substitution is present in a dimerisation domain and disrupts dimerisation.
- 15 11. A method of identifying a mammal which carries a mutated nucleic acid molecule encoding GDF-9B, said method comprising the steps of:
 - i) obtaining a tissue or blood sample from the mammal;
 - ii) isolating DNA from the sample; and optionally
 - iii) isolating GDF-9B DNA from the DNA obtained at step i) or ii);
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 - iv) probing said DNA with a probe complementary to either strand of the mutated GDF-9B DNA of SEQ ID NOs 11 or 17;
 - v) amplifying the amount of mutated GDF-9B DNA;

vi) determining whether the GDF-9B sequence DNA obtained in step v) carries a mutation associated with sterility or increased ovulation.

12. A method of identifying a mammal which carries a mutated nucleic acid molecule encoding GDF-9, said method comprising the steps of:

5 i) obtaining a tissue or blood sample from the mammal;

ii) isolating DNA from the sample; and optionally

iii) isolating GDF-9 DNA from the DNA obtained at step i) or ii);

iv) probing said DNA with a probe complementary to either strand of the mutated GDF-9 DNA of SEQ ID NO 5;

10 v) amplifying the amount of mutated GDF-9 DNA;

vi) determining whether the GDF-9 sequence DNA obtained in step v) carries a mutation associated with sterility or increased ovulation.

13. A use of a nucleic acid molecule which is complementary to either strand of the mutated DNA of SEQ ID NOs. 11 or 17 as a marker to identify a mammal carrying a mutated nucleic acid molecule encoding GDF-9B.

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14. A use of a marker as defined in claim 13 in a method for marker assisted selection of a mammal which possesses a genotype which is associated with either enhanced ovulation or sterility.

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15. A use of a nucleic acid molecule which is complementary to either strand of the mutated DNA of SEQ ID NO 5 as a marker to identify a mammal carrying a mutated nucleic acid molecule encoding GDF-9.

16. A use of a marker as defined in claim 15, in a method for marker assisted selection of a mammal which possesses a genotype which is associated with either enhanced ovulation or sterility
17. A probe capable of specifically hybridising to either strand of the mutated GDF-
5 9B DNA of SEQ ID NOS 11 or 17 under stringent hybridisation conditions.
18. A probe capable of hybridising to either strand of the mutated GDF-9 DNA of SEQ ID NO 5 under stringent hybridisation conditions.
19. A construct comprising a nucleic acid molecule as claimed in claim 1 or 2.
20. A vector comprising a nucleic acid molecule as claimed in claim 1 or 2.
- 10 21. A host cell which comprises a construct or vector as claimed in claim 19 or 20 which has been introduced therein.
22. A cell line comprising a host cell as claimed in claim 21.
23. A method of altering GDF-9 and/or GDF-9B bioactivity in a female mammal so as to modulate ovulation comprising the steps of either:
15 (a) inducing a partial immunisation response to endogenous GDF-9 and/or GDF-9B to partially reduce bioactivity thereof and enhance ovulation; or
(b) inducing a full immunisation response to endogenous GDF-9 and/or GDF-9B to substantially reduce bioactivity thereof and induce sterility.
24. A method as claimed in claim 23, wherein said immunisation response is induced
20 by administration of an antigenic composition comprising:
 - i) a GDF-9 polypeptide or a functional fragment or variant of GDF9; and/or

- ii) a GDF-9B polypeptide or a functional fragment or variant of GDF-9B;
together with a pharmaceutically or veterinarily acceptable carrier and/or diluent;
to a mammal in need thereof.
- 5 25. A method as claimed in claim 24, wherein said antigenic composition comprises a mild adjuvant to induce a partial immunisation response and induce enhanced ovulation.
26. A method as claimed in claim 24, wherein said antigenic composition comprises a strong adjuvant to induce a full immunization response and induce sterility.
- 10 27. A method as claimed in any one of claims 23 to 26, wherein said partial immunization response is induced by a short term immunization regime.
28. A method as claimed in any one of claims 23 to 26, wherein said full immunization response is induced by a long term immunization regime.
- 15 29. A method as claimed in claim 24, wherein said immunization response is induced passively by administration of antibodies raised against said antigenic composition.
30. A method as claimed in claim 29, wherein said antibodies are administered according to a short term regime to induce a partial immunization response and induce enhanced ovulation.
- 20 31. A method as claimed in claim 29, wherein said antibodies are administered according to a long term regime to induce a full immunization response and induce sterility.

32. A method as claimed in any one of claims 23, 24, 26, 28, 29, and 31, wherein said full immunization response is temporary and/or reversible and wherein said sterility induced comprises contraception.
33. A method as claimed in any one of claims 23, 24, 26, 28, 29, and 31, wherein said full immunization response and said sterility induced is permanent.
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34. A method for breeding a mammal having increased ovulation comprising the steps of:
 - a) identifying the nucleotide sequences of GDF-9 or GDF-9B carried by the female mammal it is proposed to breed from;
 - 10 b) identifying the nucleotide sequences of GDF-9 or GDF-9B carried by the male mammal it is proposed to breed from;
 - c) selecting the female and male animals that will result in progeny having the following characteristics:
 - i) a single copy of a mutated GDF-9 nucleotide sequence comprising:
 - 15 A) SEQ ID NO 5; or
 - B) a functional variant or fragment of the molecule in A); or
 - C) a sequence complementary to the molecule in A) or B); and/or
 - ii) a single copy of mutated GDF-9B nucleotide sequence comprising:
 - A) SEQ ID NOS 11 or 17; or
 - 20 B) a sequence complementary to the molecule(s) in A).

35. A method as claimed in claim 34, wherein said mammal is selected to have a single mutated copy of GDF-9 and GDF-9B.

36. A method for selecting a female mammal for breeding which possesses a genotype indicative of an increased rate of ovulation, said genotype comprising a single mutated copy of:

5 1) a mutated GDF-9 nucleotide sequence comprising:

a) SEQ ID NO 5; or

b) a functional variant of the molecule of a); or

c) a sequence complementary to the molecules in a) or b);

10 and/or

2) a mutated GDF-9B nucleotide sequence comprising:

a) SEQ ID NOs 11 or 17; or

b) a sequence complementary to the molecules in a)

15 said method comprising identifying said mammal according to the method of claim 11 and/or 12 and selecting said mammal.

37. A method as claimed in claim 36 wherein the mammal selected has both a single mutated copy of GDF-9 and GDF-9B.

38. A method of modifying the function of the corpus luteum by administering supplementary GDF-9 or GDF-9B , or analogues thereof, or GDF-9 or GDF9-B antagonists to female mammals.

39. A transgenic non-human animal wherein one copy of the endogenous GDF-9 and/or GDF-9B gene has been knocked out.
40. A transgenic non-human animal as claimed in claim 39, comprising a transgenic ovine having a genome lacking one copy of a gene encoding a protein having biological activity of GDF-9 and/or GDF-9B.
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41. An isolated mutated GDF-9 polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 3, or 6 or a functional fragment or variant thereof.
42. An isolated mutated GDF-9B polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 10, 12, 14, 16, or 18.
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43. A composition comprising an isolated nucleic acid as claimed in any one of claims 1 to 10, or an isolated polypeptide as claimed in claim 41 or 42 and a pharmaceutically acceptable carrier.